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14. ABSTRACT

The purposes of this study are to provide quantitative estimates of 1) the effective amount of vitamin D produced in the skin as a function of skin pigmentation; and 2) the rate of utilization of vitamin D as a function of ethnicity. The outcome will be estimates of the amount of vitamin D that must be given orally to military personnel of different races and in different assigned locations so as to ensure and maintain normal vitamin D status. In the first 39 months' work (the period covered by this report), we have accumulated 80+% of the targeted specimens for both objectives, in a racially diverse sample. In addition we have augmented our findings from naturally sun-exposed individuals to include responses in volunteers receiving controlled doses of UV-B. Analyses are continuing and will be completed within the coming months. No final quantitative results will be available until all the measurements have been made and analyzed as a unit.

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Vitamin D, race, skin color

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## **INTRODUCTION**

The purpose of this project is to develop quantitative estimates of 1) the amount of vitamin D produced by skin exposure to sunlight (Experiment 1, below), and 2) the amount of oral vitamin D that must be given to supplement solar inputs so as to achieve desired vitamin D levels in military personnel of differing races and skin pigmentation (Experiment 2, below).

This is the fourth annual report with respect to the above-referenced award. Although the award was made as of 1 October 2001, authorization to proceed was not received from USAMRMC until 15 July 2002. Hence this report, although technically covering the first four years of the award, describes work performed only from 15 July 2002 until submission of this report, i.e., a period of little more than three years.

## **BODY OF REPORT**

Logistics. As noted in prior reports, this project depends strongly upon a project manager with a minority background and good community contacts. We described how we "went through" a series of these managers, each leaving us for better jobs at crucial times in the project. We have compensated by falling back on core staff at our Center and by shifting to other strategies to help us reach our goals.

Work Performed to Date: Experiment 2. The purpose of Experiment 2 is to quantify the serum 25(OH)D response (and its physiological correlates) to summer sun exposure in persons with a wide range of skin pigmentation. As of 30 September 2005 we had enrolled 79 individuals and had obtained both the first (i.e., late summer) and the second (late winter) measurements for most of them, as specified for Experiment 2. This number (79) is just shy of our target of 80 participants with the shortfall being due to the loss of a project manager in two successive years at the peak of late summer recruitment. The racial and sex breakdown of the group so far recruited into Experiment 2 is as follows:

	Non-Hispanic Caucasian	Hispanic	African-American	Totals
Male	18	6	23	47
Female	13	2	17	32
Totals	31	8	40	79

For each of these subjects we have obtained (or are in the process of obtaining) the suite of specimens/measurements specified in the approved protocol, i.e., history of sun exposure by duration and clothing type; skin pigmentation by reflectance meter measurement; calcium absorption efficiency; measurements of the full set of hormones regulating the calcium economy [i.e., PTH, 1,25(OH)<sub>2</sub>D<sub>3</sub>, 25(OH)D], as well as blood vitamin D levels themselves, urine calcium excretion, and bone densitometry. While degree of sun exposure varied, all participants were selected because they self-reported substantial mid-day sun exposure throughout most or all of the summer.

As noted previously, we have sufficient data to permit several observations. Many of them (e.g., black-white differences) were already known from other studies; our goal in this project was to quantify them so as to develop better estimates of the amount of vitamin D producible in the skin in persons of varying color. This will allow (along with results of Experiment 1, below), development of evidence-based guidelines for vitamin D supplementation of DoD personnel. Findings to date (based on data from 65 subjects) are as follows:

- African-Americans have lower serum 25(OH)D values than whites at both summer and winter measuring points. Although we have too few Hispanic subjects for a precise estimate, their data tend to be intermediate between blacks and whites.
- African-Americans, working outdoors, elevate their serum 25(OH)D levels to an extent not clearly different from whites (based on data available to date).
- Neither serum calcium nor urine calcium excretion differs between the late summer and late winter measurement times.
- Serum PTH rises significantly from late summer to late winter, indicating that the late winter level of 25(OH)D is physiologically inadequate (thus evoking increased PTH secretion).
- Calcium absorption efficiency is slightly (but significantly) higher at the late summer measurement point. This finding, as with the PTH difference, indicates that, by late winter, there is not only chemical evidence of vitamin D deficiency (i.e., low serum 25(OH)D), but physiological evidence as well (i.e., lower intestinal calcium absorption). Thus, steps to correct this inadequacy are likely to produce a benefit.

The recruitment problem we have had for this component of the project is partly a reflection of national experience in recruiting persons of color into any clinical research study, and partly due to the fact that the outdoor workers could not easily afford losing a day's work (and pay). To compensate for this recruitment shortfall, we have devised a strategy that expands the pool of subjects and also provides a better quantification of UV-B exposure.

This revised strategy uses a dermatologist's UV light box (National Biological HOUVA II UV Light Booth, National Biological Corp., Twinsburg, OH), delivering calibrated whole body exposure to selected UV-B light intensities, measured in mJoules. (For reference purposes, 15 minutes of outdoor sun exposure at our latitude in July at mid day is 20 mJ.) Volunteers receive 3× weekly radiation. Serum 25(OH)D level is followed for 8 weeks, and analyzed as a function of constitutive skin tone. Skin tone, described in previous reports, is measured using an IMS Smart-Probe 400 (IMS, Inc., Milford, CT), which distinguishes the three principal components of skin color: black (melanin), yellow (carotene, etc.), and red (blood flow). The melanin component absorbs UV-B and reduces its ability to make vitamin D on sun exposure. "Constitutive" skin color is given by the melanin level on typically unexposed skin (e.g., the inside surface of the upper arm).

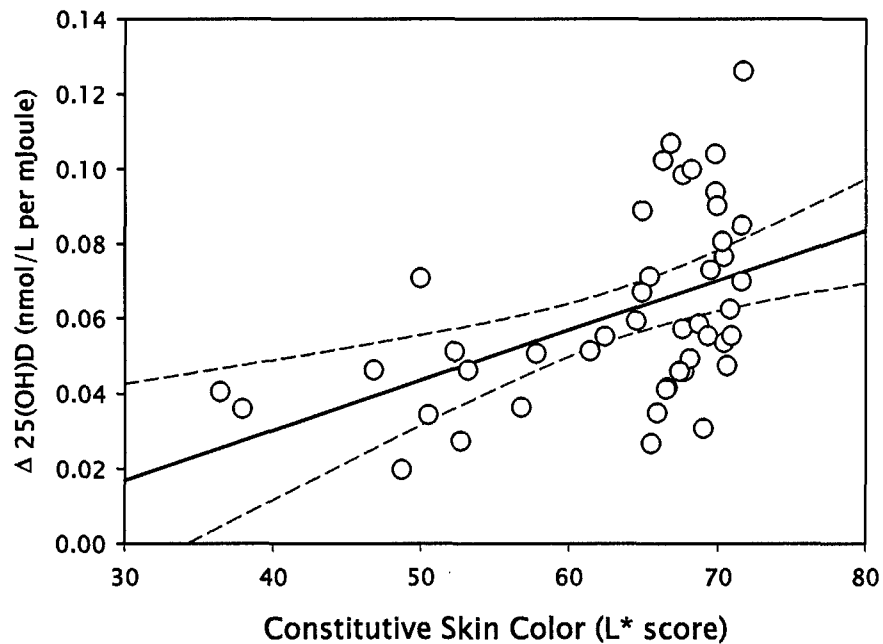


Fig 1. Change in serum 25(OH)D produced by UV-B radiation, expressed as a function of constitutive skin color. The exposure referred to in the vertical axis is the number of mJ per session, and the rises in serum 25(OH)D are those measured after four weeks of thrice weekly exposure.

As would be predicted, 25(OH)D response to UV-B exposure is inversely proportional to constitutive skin color. Fig. 1 presents a portion of the data analyzed to date, showing the change produced in serum 25(OH)D, per mJ of UV-B in 48 subjects of varied skin color, expressed as a function of constitutive skin tone. The horizontal axis is the  $L^*$  scale value of skin color, i.e., a measure of blackness (low values) and whiteness (high values). As would have been expected, each mJ of UV-B produces a greater rise in serum 25(OH)D in light-skinned persons than in dark. Our purpose in this aspect of the project was not to find what was already known (i.e., response is a function of skin color), but to quantify it – i.e., to define how much sun exposure is needed to produce desired 25(OH)D values, and to do so as a function of inherent skin color. Results, when combined with the findings of Experiment 1, will translate to the oral dosing of vitamin D needed to achieve the desired serum 25(OH)D values irrespective of race or color.

Work Performed: Experiment 1. The purpose of Experiment 1 is to quantify the ethnic differences (if any) in metabolism of known inputs of vitamin  $D_3$ . It is designed to be executed over the winter months when solar vitamin D input is minimal and total input can be controlled by the investigators through daily oral dosing of controlled quantities of vitamin  $D_3$ . Our plan was to split the project into two phases, studying doses of zero and 1000 IU/d during the first year of the project, and doses of 5,000 and 10,000 IU/d during the second year. We have completed specimen acquisition for this Experiment, and have completed the biochemical analyses. Data analysis is deferred until all data have been compiled. The basic numbers were reported in last year's report.

Racial and sex distribution of participants in Experiment 1 is as follows:

	Non-Hispanic Caucasian	Hispanic	African-American	Totals
Male	5	16	3	24
Female	13	9	48	70
Totals	18	25	51	94

These numbers are above our target figure of 80 enrollees.

Preliminary analysis of the data indicate that African American metabolism of vitamin D is similar to that of Caucasians.

Work Plan for the Forthcoming Year. We have set a tentative target of 15 additional individuals in the summer sun exposure phase of the project (Experiment 2), seven of whom have by the date of this report completed the first (summer) study of the paired studies for Experiment 2. This will take us well above our original enrollment targets, but is considered necessary since detailed questioning of several of the previously enrolled outdoor workers revealed little actual sun exposure in several of them. We anticipate spending most of the coming year on this additional work and on completion of analysis of acquired specimens, on data clean-up, specimen reanalysis (as needed), statistical modeling and analysis, and report generation.

### **KEY RESEARCH FINDINGS**

Key research findings (on the still incomplete dataset) are as set forth under Experiments 2 and 1, above. In addition to the presentation described in prior years' progress reports, a portion of these results were presented in poster form at the meeting of the American Society for Bone and Mineral Research in Nashville, TN, September 24, 2005. Copies of the poster and the associated Abstract are attached as Appendix I. Full analysis and publication must wait completion of analyses for all the subjects during this coming year.

### **REPORTABLE OUTCOMES**

As noted in the foregoing, the reportable outcomes from this study will consist of 1) best quantitative estimates of skin production of vitamin D as a function of skin pigmentation and extent of skin exposure; and 2) best quantitative estimates of rate of utilization of vitamin D<sub>3</sub> as a function of race/ethnicity. Taken together, both will yield estimates of the quantity of vitamin D that must be given to military personnel to ensure maintenance of desired vitamin D status. Since much of the work is still underway, final quantitative estimates are not yet available. However, from the data produced so far, it seems safe to say that we will be able to produce the projected quantitative estimates. Moreover, secondary findings will become available and doubtless further such will develop as we accumulate more measurements. An example of such secondary data can be found in the Abstract and Poster attached as Appendix I.

### **CONCLUSIONS**

None to date except as described above from partial analysis of the sample.

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2. Armas LAG, Heaney RP, Barger-Lux MJ, Huerter C, Lund R. The effects of UV-B light on serum 25(OH)D in humans. *J Bone Miner Res* 20 (Suppl 1):S188, 2005.



***APPENDIX I***

## **The Effects of UV-B Light on Serum 25(OH)D in Humans**

L. A. G. Armas\*<sup>1</sup>, R. P. Heaney<sup>1</sup>, M. J. Barger-Lux\*<sup>1</sup>, C. Huerter\*<sup>2</sup>, R. Lund<sup>3</sup>. <sup>1</sup>Osteoporosis Research Center, Creighton University, Omaha, NE, USA, <sup>2</sup>Dermatology, Creighton University, Omaha, NE, USA, <sup>3</sup>Nephrology, Creighton University, Omaha, NE, USA.

**Presentation Number:** SA519

We report results of work to quantify the relationship of skin color and 25(OH)D response to graded doses of UV-B light delivered by a light booth. The subjects (n=48, age 21-49 yr, females = 28, males = 20) were healthy indoor workers with limited non-solar sources of Vitamin D. They were divided into 5 treatment groups based on their self-reported susceptibility to tan or burn (Fitzpatrick skin types I-VI).

Data were gathered from January through April. We determined BMI, 25(OH)D, Ca<sup>2+</sup> and PTH at baseline. We used a portable skin colorimeter that utilizes the CIE L\*a\*b\* color system to measure constitutive skin color of the upper inner arm and facultative skin color of the forearm. The subjects were exposed to UV-B light from a UV light booth 3 times a week for 4 weeks (12 treatments) in graded doses ranging from 40mJ to 80 mJ per treatment. 25(OH)D was drawn weekly during, and 4 weeks after, completion of UV-B treatment.

There was a rise in 25(OH)D of 29.9 nmol/L (median; interquartile range 24.3-40.7) during the 4 weeks of UV-B treatment and a fall 4 weeks after UV-B treatment ceased of 3-14% from peak 25(OH)D levels. The L\* values of exposed skin did not vary significantly throughout the treatment period. There was a significant correlation between L\* readings (a continuous darker-to-lighter scale) of baseline facultative and constitutive skin color and 25(OH)D response per mJ of UV-B light given ( $r^2=0.535$ ,  $r^2=0.551$ ) (i.e. the lighter skinned subjects had a greater response). In conclusion, increase in 25(OH)D per mJ of UV-B exposure was related to the "L" value of skin at baseline. This increase in 25(OH)D was achieved without changing "L\*" values (i.e. becoming darker).

# Effect of UV-B Light on Serum 25(OH)D in Human

Michael F. Holick, M.D., Ph.D., Harvard Medical School, Boston, Massachusetts; University of Massachusetts, Lowell, Massachusetts

## METHODS continued

Parameter	Value
Number of subjects	10
Age (mean)	32
Sex (M/F)	5/5
Weight (mean)	70
Height (mean)	170
25(OH)D (mean)	25
25(OH)D (range)	10-40

Parameter	Value
Number of subjects	10
Age (mean)	32
Sex (M/F)	5/5
Weight (mean)	70
Height (mean)	170
25(OH)D (mean)	25
25(OH)D (range)	10-40

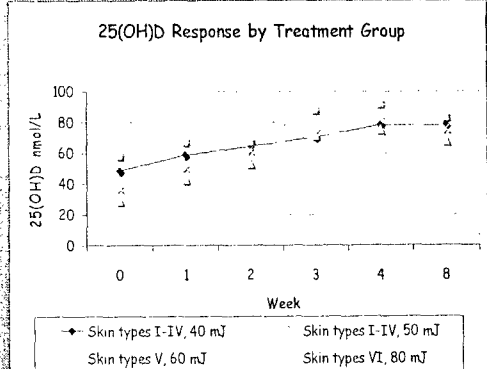
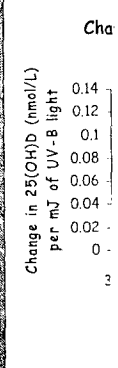


Chart 1 displays median 25(OH)D levels by treatment group throughout the study.



Graph 2 displays the change in 25(OH)D levels by treatment group throughout the study.

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# 5(OH)D in Humans

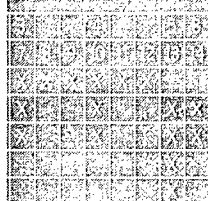
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5(OH)D levels by treatment group

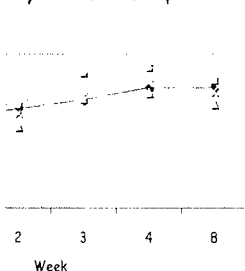


5(OH)D levels by treatment group

Week	2	3	4	8
Mean	3.2	3.5	3.8	4.1
SD	1.2	1.3	1.4	1.5
95% CI	(1.8, 4.6)	(2.2, 4.8)	(2.4, 5.0)	(2.6, 5.2)
Median	3.1	3.4	3.7	4.0
Range	(1.8, 4.6)	(2.2, 4.8)	(2.4, 5.0)	(2.6, 5.2)
Mean	3.2	3.5	3.8	4.1
SD	1.2	1.3	1.4	1.5
95% CI	(1.8, 4.6)	(2.2, 4.8)	(2.4, 5.0)	(2.6, 5.2)
Median	3.1	3.4	3.7	4.0
Range	(1.8, 4.6)	(2.2, 4.8)	(2.4, 5.0)	(2.6, 5.2)

5(OH)D levels by treatment group

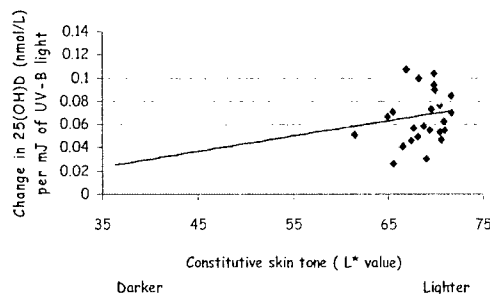
by Treatment Group



5(OH)D levels by treatment group

5(OH)D levels by treatment group

Change in 25(OH)D per mJ of UV-B light



Graph 2 demonstrates the correlation between constitutive skin tone by L\* value and change in 25(OH)D per mJ of UV-B light. \*Spearman's R (2-tailed significance) P < .0001

RESULTS continued

Skin Tone	1	2	3
Femiles	darkest	mid	lightest
Change in 25(OH)D per mJ of light	0.046	0.057	0.073
baseline 25(OH)D nmol/L	37.6	44.9	48.5
	(26.7, 50.2)	(41.9, 61.6)	(43.6, 62.2)

There was a significant difference in the change per mJ of UVB light between the darkest and lightest femiles. There was a significant difference between baseline 25(OH)D levels between the darkest and lightest femiles. Wilcoxon signed-ranks test showed no significant difference between exposed skin tone (flank) at baseline and after 4 weeks of UV-B exposure.

DISCUSSION

Increase in 25(OH)D per mJ of UV-B exposure was related to the constitutive skin color. Those with the lightest skin tones produced more 25(OH)D per mJ of light exposure. The increase in 25(OH)D levels was achieved without changing exposed skin tone (i.e. becoming darker or tanning).

1. Supported by grants from Dialysis Clinical, Inc., The Endocrine Fellows Foundation and by research funds of Creighton University.
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